Cytotoxicity and Corrosion of Carbide Derived Carbon (CDC) coated Titanium Implants in infectious Environment

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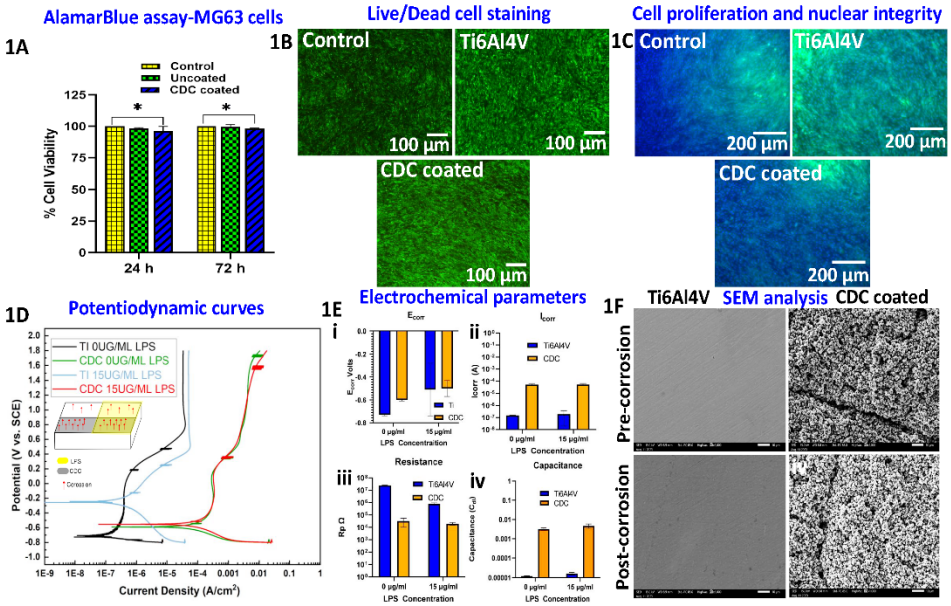
INTRODUCTION: Total Hip Replacements (THR) are widely accepted in Orthopedic treatment and clinical management. In 2009, metal-on-metal (MoM) THRs were recalled due to serious complications arising from wear and corrosion. As a solution, carbide-derived carbon (CDC) coating is being investigated as a promising way to improve the lifespan of MoM hip implants. In fact, the CDC is inspired by the graphitic carbon tribolayer which naturally occurs on metal implants [1]. Due to its similarity with the tribo-layer, we expect CDC will be produced during normal implant function giving the coating a self-healing quality. The CDC layer provides lubrication and has the potential to minimize the wear and corrosion of MoM hip implants without damaging surrounding tissues. We previously reported the CDC on CoCrMo [1]. Our present study focuses on the corrosive behavior of CDC-coated titanium alloy (Ti6Al4V) in the presence of an infectious environment simulated using Lipopolysaccharides (LPS) [2]. We hypothesize that the CDC-coated Ti implant will have optimized cytotoxicity and comparable corrosion resistance in the body environment. Our expected outcome is that, since Ti is widely used in implants, this study will provide more insight into exploring better and safer orthopedic devices.

METHODS: We are comparing two base materials 1) Titanium alloy Ti6Al4V and 2) Ti6Al4V coated with CDC. The groups are named Ti6Al4V and CDC respectively. Ti6Al4V samples are polished with standard metallographic methods to achieve a mirror finish with a surface roughness of <50nm. CDC samples are prepared using a methodology proposed by Sun *et al.* [3]. In short, there is first a carburization process that occurs at high temperatures under an inert gas atmosphere. The carburization is followed by the deposition of the CDC layer using electrolysis in molten salt. **(i) *In vitro* cytotoxicity:** *In vitro* cytotoxicity studies were performed using MG63 cells. The samples were tested for cell viability using AlamarBlue for the duration of 24 and 72 h. Live/Dead cell staining and cell proliferation and nuclear integrity by FITC-DAPI staining for the duration of 72 h. ***(ii) Corrosion Analysis:*** After sample preparation, we compared the corrosion behavior in two solutions. 1) Bovine Calf Serum 2) Bovine calf serum with the LPS (15 µg/mL). Corrosion resistance is analyzed using a 3-electrode electrochemical cell, as per ASTM G61. Our sample is the working electrode, graphite is used as a counter electrode, and a saturated calomel electrode (SCE) is used as the reference electrode. The electrochemical sequence can be listed as; 1. Open circuit potential, 2. Cyclic polarization test 3. Electrochemical Impedance Spectroscopy test. After plotting the polarization curves, using Tafel's method, Corrosion potential, $E_{corr,}$ and corrosion current, I_{corr} were estimated. EIS data is used to construct Nyquist and Bode plots and an equivalent electrical circuit is developed to determine the polarization resistance and capacitance. After testing, the corroded surfaces were examined using white light profilometry and scanning electron microscopy.

RESULTS:*(i) In vitro cytotoxicity:* The *In vitro* cytotoxicity studies by AlamarBlue assay with MG63 cells studies showed comparable cell viability with CDC-coated Ti samples **(Fig 1A)**. Similarly, the Live/Dead cell staining study showed good cell viability **(Fig 1B).** Cell proliferation and nuclear integrity of the samples showed better cell proliferation and nuclear integrity with CDC-coated Ti samples than uncoated Ti samples (**Fig 1C**). ***(ii) Corrosion analysis:*** The Potentiodynamic curves are presented in **Fig.1D**, which shows the typical passivation behavior expected from the Ti surfaces. For the Ti6Al4V Tafel parameters, the presence of LPS increased the average I_{corr} by 30% and decreased the E_{corr} by 30%. This is compared to the CDC samples where the average I_{corr} increased by <1% and the E_{corr} decreased by 15% (see **Fig.1E**) Without LPS, Ti samples showed slightly higher E_{corr} values and lower I_{corr} values. After adding LPS, CDC & Ti samples had similar average E_{corr} values and I_{corr} values remained lower for the uncoated Ti samples. Electrochemical Impedance Spectroscopy (EIS) results in **Fig 1E**: Without the presence of LPS, CDC samples had an average Rp of $3.2x10^6$ Ω, while Ti samples had an average Rp of $1.3x10^7$ Ω. With the addition of LPS the Ti group showed a 1000-fold decrease in resistance, while the CDC group changed by less than 2x. Capacitance values, Yo, were higher for the CDC by 2 orders of magnitude with and without LPS. Both surfaces showed a modest increase in capacitance between the 0 µg /mL and the 15 µg /mL BCS solutions. Surface characterization **Fig 1F**: When comparing the pre-corrosion and post-corrosion surfaces via SEM imaging neither material showed significant corrosion damage.

DISCUSSION: The study indicates the cytotoxicity of CDC is comparable with uncoated Ti, and acceptable for the implant application. While corrosion properties in this experiment were better for uncoated titanium alloy the CDC dampened the corrosive effects of LPS. EIS results pointed to a lower resistance and higher capacitance in the CDC samples. The polarization curves similarly showed CDC samples had a lower E_{corr} and higher I_{corr} suggesting increased propensity and rate of corrosion. The protective quality of CDC coating was demonstrated by the minimal change in the behavior of the CDC samples with and without the presence of LPS while the titanium samples showed significantly increased corrosion. This finding suggests that the CDC coating is protective in the corrosive LPS environment, to some extent. This experiment sets up the framework for our future experiments using mechanical articulation for tribological simulation. As indicated by our previous study, we expect the CDC will provide superior lubrication and chemical protection against de-passivation. Future research will be combined with results from these corrosion experiments to better elucidate the mechanism of protection in the presence of corrosive environments such as bacterial LPS.



SIGNIFICANCE: CDC coatings may provide the mechanical and chemical protection needed to address current challenges in using Metal-on-Metal hip implants. With optimized cytotoxicity and acceptable corrosion resistance, the CDC could be considered as a protective coating for implant applications in order to minimize infection-associated damage and create safer implants for the orthopedic community.

ACKNOWLEDGEMENTS: James Scholar program at Office of Research (UICOM-R).

REFERENCES:[1] Y. Liao *et al.* [2] M. T. Mathew *et al.* [3] Y. Sun *et al.*

The major findings of the study include Figure 1: **A -** *In vitro,* cell viability with MG63 cells using AlamarBlue assay showed good cell viability. **1B -** Live/Dead cell staining showed higher cell viability. **1C** - FITC-DAPI staining showed good cell proliferation with nuclear integrity. **1D -** Potentiodynamic studies showed good corrosion resistance of CDC coated samples. **1E -** After adding LPS, CDC & Ti samples showed average E_{corr} values and I_{corr} values and lower for uncoated samples. **1F-** SEM analysis showed no significant corrosion in either sample